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Plasmid profile of *Neisseria gonorrhoeae* in Nigeria and efficacy of spectinomycin in treating gonorrhoea

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SUMMARY The prevalence of penicillinase producing *Neisseria gonorrhoeae* (PPNG) strains has been steadily rising in Nigeria since 1979, and now about 80% of the strains of gonococci isolated in Ibadan are found to produce penicillinase. Spectinomycin has consequently become widely used in treating these infections. To ascertain the emergence of spectinomycin resistance, this study was undertaken to assess the in vivo susceptibilities of gonococcal strains to spectinomycin and other common antibiotics. Five hundred and twenty seven isolates were tested, of which 452 (85·5%) were PPNG strains. None of the strains were found to be resistant to 100 μg spectinomycin discs in vitro, whereas all 370 patients treated with the antibiotic were bacteriologically cured. Plasmid analysis shows that both "Asian" and "African" PPNG types are circulating in Nigeria. For the moment spectinomycin remains highly effective in treating gonococcal infections in west Africa.

Introduction

There has been great variation in the sensitivity of the gonococcus to antibiotics throughout the world during the past decade. The penicillinase producing strains of *Neisseria gonorrhoeae* (PPNG) have provided a useful marker for studies of the spread of isolates resistant to antibiotics, particularly in the Far East and African countries. For many years in Nigeria there has been an overall trend to reduction in sensitivity of gonococci to penicillin, ampicillin, and tetracycline, which has been associated with environmental selective pressures and the improper use of antibiotics.[1] The increasing cost of alternative drugs, self medication, and lack of adequate treatment strategies have provided the epidemiological setting conducive to the spread of penicillinase producing strains of *N gonorrhoeae*. The recognition of spectinomycin resistant PPNG strains[2] and an increasing number of non-PPNG strains highly resistant to penicillin and other antimicrobials provide cause for concern.

In Nigeria the incidence of infections with PPNG strains has steadily increased since the first isolates were identified locally in 1979, when the incidence was 2·7%.[3] Within two years this figure had increased dramatically to 51·7%.[4] In contrast, a survey of the incidence of PPNG strains in the United Kingdom in 1979–80[5] showed an annual exponential increase with the evolution of endemic spread of infection and epidemiological evidence of an appreciable proportion of infections still being acquired overseas. Most were imported from South East Asia (50%) and west Africa (34%). In 1980 in the United Kingdom the prevalence of PPNG strains was low (fewer than 1%), but subsequently in some urban areas it approached 5%. The origin of these PPNG strains was correlated with penicillin resistance plasmids of two types: a 4·4 megadalton plasmid with or without an additional conjugative plasmid (24·5 megadalton) originating in South East Asia, and a 3·2 megadalton plasmid originating in west Africa.[6] Many workers have used the molecular biological characteristics of these strains to identify isolates as being of "Asian" or "African" origin on the basis of plasmid size and to assess the possible geographical source of infection. More recent investigations suggest that labelling PPNG strains as "Asian" or "African" is no longer valid.[7]

New plasmid patterns have been identified in Africa and Europe,[7-10] and multiresistant PPNG strains have spread throughout Africa.[11] With the advent of penicillinase producing gonococci and their subsequent global spread spectinomycin was introduced as a

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first line drug, but there have been disturbing reports of the emergence of spectinomycin resistant gonococcal isolates elsewhere in areas of high prevalence.[12] This report examines the efficacy of spectinomycin in treating gonorrhoea caused by PPNG strains in Nigeria in 1982–4 and studies of the antibiotic sensitivities and molecular characterisation of the plasmids of these strains.

Patients, materials, and methods

STRAINS

Culture of 527 gonococcal isolates obtained from patients attending the Special Treatment Centre in Ibadan yielded 452 PPNG strains. The criteria for diagnosing gonorrhoea were the identification of Gram negative intracellular diplococci in a smear of urethral, cervical, or vaginal exudate, and culture on Thayer-Martin selective medium. The culture plates were incubated in candle extinction jars at 37°C for 24-48 hours. Gonococcal strains were identified and confirmed by conventional methods.[13] All gonococcal isolates were screened for penicillinase production in the department of medical microbiology, University College Hospital, Ibadan, using 6 μg ampicillin discs, and β lactamase was detected by the chromogenic cephalosporin (Nitrocefin) test.[14] Sensitivities to antibiotics were measured using disc diffusion tests for penicillin, tetracycline, chloramphenicol, streptomycin, and spectinomycin: 100 μg spectinomycin discs were used for susceptibility to spectinomycin, and isolates that gave an inhibition zone of more than 20 mm in diameter were regarded as sensitive.

Surveillance of PPNG strains

Forty nine PPNG isolates were randomly selected in 1983–4 and sent to the London Hospital sexually transmitted diseases (STD) reference laboratory for assessment of antibiotic sensitivities and molecular characterisation. On reconstitution, however, only 27 isolates had survived transportation. These strains were screened again for penicillinase production and processed for antimicrobial susceptibility and plasmid typing.

ANTIBIOTIC SENSITIVITY TESTS

The minimum inhibitory concentrations (MICs) of penicillin, tetracycline, streptomycin, cefuroxime, kanamycin, and spectinomycin were assessed by the agar plate dilution method using brain heart infusion agar (BH/G/A) free from antibiotics and enriched with 1% chemically defined supplement (Gibco) and 10% defibrinated horse blood, which was prepared in the London Hospital STD reference laboratory. Two international (World Health Organisation) reference strains with known sensitivity patterns were tested simultaneously in each batch.

Antibiotics

Reagent solutions of pencillin, tetracycline, streptomycin, kanamycin, spectinomycin, and cefuroxime were prepared and dilutions added to the medium (BH/G/A) to give the required final concentrations of penicillin (0·004-100 mg/l), tetracycline (0·06-4 mg/l), spectinomycin (10-40 mg/l), cefuroxime (1-2 mg/l), streptomycin (10-500 mg/l), and kanamycin (2-10 mg/l). The inoculum size was standardised to a concentration of about 10^8 organisms. A 100-fold dilution of each suspension was transferred to the appropriate antibiotic containing plate using a Steers multipoint replicator. After incubation for 24 hours the MIC was assessed as the lowest concentration of antibiotic that completely inhibited growth of the strains tested. Gonococcal strains inhibited by 0·5 mg/l or more penicillin G were classified as resistant.

ISOLATION AND IDENTIFICATION OF PLASMIDS

All β lactamase producing isolates were subcultured on BH/G/A medium supplemented with 1 mg/l ampicillin. Plasmid DNA was identified by agarose-gel-electrophoresis using the methods described by Eckhardt[15] and Kado and Liu.[16] Reference strains with plasmids of known molecular weight were included as standards. The plasmid profiles shown were identified as being African, Asian$^-$, or Asian$^+$ (without or with the 24·5 megadalton plasmid).

TREATMENT AND TEST OF CURE

In this study 370 patients were treated with an intramuscular injection of 2 g spectinomycin in the gluteal region, and were advised to refrain from sexual contact and alcohol during follow up. The patients were examined 3, 8, and 21 days after treatment. The criteria used for bacteriological cure were the absence of gonococci on Gram stained smear microscopy and negative cultures. Subjects with positive cultures usually between three and 14 days after treatment were considered to be treatment failures.

Results

We tested 527 gonococcal isolates in Ibadan, and these were sensitive to spectinomycin (100 μg) by disc diffusion. PPNG strains accounted for 452 (85·8%), and 75 (14·2%) were non-PPNG strains, which is consistent with the increased incidence of penicillin resistant gonococcal isolates identified in Nigeria since 1979 (table I). In the study published here no spectinomycin resistant gonococci were detected by disc tests, and subsequently in the London Hospital STD reference laboratory none of the isolates was found to have MICs of spectinomycin of more than 30 mg/l. Table II shows the distribution of the MICs

TABLE I *Results of screening gonococcal isolates for β lactamase production in Ibadan, Nigeria, 1977-84*

Year	*No of gonococcal isolates screened*	*No (%) of PPNG strains found*
1977	80	0
1978	No screening	
1979	257	32 (12·5)
1980	238	53 (22·3)
1981	304	152 (50·0)
1982	460	323 (70·2)
1983	422	320 (75·8)
1984	406	329 (81·0)

and the plasmid composition of some of these PPNG isolates acquired by Africans in Nigeria. The plasmid profile of 27 randomly selected strains showed 10 Asian$^+$, four Asian$^-$, and 13 African types. Table III shows an analysis of a limited number of Nigerian PPNG strains by serogrouping using monoclonal reagents (Phadebact) against WI and WII/III serogroups. The sample was small, but serogroup WII/III predominated. In the therapeutic follow up study of 370 patients (272 men and 98 women) harbouring β lactamase producing gonococci, 30 defaulted and three were reinfected during follow up, leaving 337 patients who were assessed. The overall cure rate was 100%. Thirty five (13%) men developed post-gonococcal urethritis and responded to a seven day course of tetracycline. Treatment with spectinomycin produced no adverse side effects, and patient tolerance was good.

TABLE III *Plasmid typing and serogrouping of 15 penicillinase producing strains of Neisseria gonorrhoeae from Nigeria*

Plasmid molecular mass (megadaltons)	*No of strains*	*Serogroups:*	
		WI	*WII/WIII*
2·6 3·2	10	5	5
2·6 4·4 24·5	4		4
2·6 4·4	1	1	
Total	15	6	9

TABLE II *Minimum inhibitory concentrations (MICs) of six antimicrobial agents for 27 penicillinase producing Neisseria gonorrhoeae (PPNG) strains according to plasmid types*

Strain No	*Plasmid molecular mass (megadaltons)*	*Plasmid type*	*MICs (mg/l) of:*					
			Penicillin	*Tetracycline*	*Spectinomycin*	*Cefuroxime*	*Kanamycin*	*Streptomycin*
1	24·5 4·4 2·6	Asia$^+$	> 100	4	30	1	8	> 500
2	24·5 4·4 2·6	Asia$^+$	> 100	2	30	1	8	> 500
3	24·5 4·4 2·6	Asia$^+$	> 100	4	30	2	10	> 500
4	24·5 4·4 2·6	Asia$^+$	> 100	4	30	1	8	> 500
5	24·5 4·4 2·6	Asia$^+$	> 100	4	30	2	10	> 500
6	24·5 4·4 2·6	Asia$^+$	> 100	4	20	1	4	> 500
7	24·5 4·4 2·6	Asia$^+$	> 100	4	20	1	10	> 500
8	24·5 4·4 2·6	Asia$^+$	> 100	2	25	1	10	> 500
9	24·5 4·4 2·6	Asia$^+$	> 100	4	30	2	8	> 500
10	24·5 4·4 2·6	Asia$^+$	> 100	2	30	1	10	> 500
11	4·4 2·6	Asia$^-$	> 100	4	30	1	8	> 500
12	4·4 2·6	Asia$^-$	> 100	1	30	1	8	> 500
13	4·4 2·6	Asia$^-$	> 100	4	30	1	8	> 500
14	4·4 2·6	Asia$^-$	25	1	20	1	8	10
15	2·6 3·2	Africa	50	2	30	1	4	> 500
16	2·6 3·2	Africa	50	2	20	1	4	> 500
17	2·6 3·2	Africa	50	4	30	1	8	> 500
18	2·6 3·2	Africa	100	1	20	1	8	10
19	2·6 3·2	Africa	> 100	2	25	1	8	> 500
20	2·6 3·2	Africa	> 100	1	25	1	8	> 500
21	2·6 3·2	Africa	> 100	1	25	1	8	> 500
22	2·6 3·2	Africa	> 100	1	25	1	10	> 500
23	2·6 3·2	Africa	> 100	1	25	1	10	> 500
24	2·6 3·2	Africa	> 100	2	25	1	10	> 500
25	2·6 3·2	Africa	> 100	2	25	1	10	> 500
26	2·6 3·2	Africa	> 100	4	30	1	10	> 500
27	2·6 3·2	Africa	> 100	2	30	1	10	> 500

All strains tested were sensitive to spectinomycin but resistant to tetracycline (≥ 1 mg/l) and streptomycin (≥ 500 mg/l) and relatively insensitive to kanamycin (8-10 mg/l).

Discussion

PPNG strains present a serious problem in many parts of the world. There is urgent need for alternative effective and inexpensive treatment to combat the threat provided by PPNG strains and non-PPNG strains with reduced sensitivity to antimicrobials. In 1982 the incidence of PPNG strains increased rapidly in west Africa,[4] and the prevalence (85%) in the study published here compares with reports from other parts of the country (Ilorin 74·2%, Enugu 73%, personal communication).

Both African and Asian type PPNG strains are thought to be well established and circulating freely throughout African countries, and the high levels of incidence identified in Nigeria are remarkable when compared with those of other areas of high incidence, such as the Philippines (30-40%),[17] Thailand (42%),[18] Japan (16·1%),[19] Singapore (33·5%),[20] Indonesia (25%),[21] and the Netherlands (where there was regional variation in incidence of 4-25% of all gonococcal isolates.[7 22] The dramatic increase of infections with PPNG strains in the Netherlands coincided with the emergence of a hybrid variant of the African strain that had acquired the large 24·5 megadalton conjugative plasmid in addition to the small 3·2 megadalton plasmid, which promoted greater stability and apparent selective advantage of these isolates within local gonococcal populations. Such isolates have not been documented in PPNG strains isolated in Nigeria, but eight have been identified in the STD reference laboratory of the London Hospital from patients with primary source contacts in Nigeria and Zambia (unpublished data).

In many African countries prostitution is common, there is widespread indiscriminate use of oral antibiotics, and the lack of control of the sale of antibiotics promotes high rates of treatment failure. Our studies confirmed that African PPNG strains are highly resistant to penicillin (MICs $\geq$ 100 mg/l), tetracycline (MICs 1-4 mg/l), and streptomycin (MICs $\geq$ 500 mg/l), and are also relatively insensitive to kanamycin (table II). In contrast, all strains tested were susceptible to spectinomycin and cefuroxime, but it is noteworthy that the MICs for spectinomycin and cefuroxime were found at the upper range of tolerance to antibiotics that is acceptable for treating gonorrhoea.

Though spectinomycin resistant PPNG strains are reported rarely throughout the world,[2 12] nevertheless in Britain in 1982-3 at least 50 spectinomycin resistant PPNG strains were identified, mainly in the London area, and 28 of these were referred to and confirmed in the London Hospital STD reference laboratory. All the strains carried plasmids of 4·4, 2·6, and 24·5 megadaltons. So far there is no documented evidence of spectinomycin resistance associated with the treatment of penicillinase producing *N gonorrhoeae* in Nigeria, but with the routine use of this antibiotic similar therapeutic problems may arise. It is purely conjectural whether and when multiple resistance to drug treatment will appear in gonococci.[23]

Spectinomycin is readily available in west Africa, though more expensive than penicillin. It is still highly effective against PPNG strains, as shown by this study. Every effort should be made to contain further spread of PPNG strains across tropical Africa by epidemiological surveillance and controlled trials re-evaluating current treatment regimens. In this way the efficacy of alternative antimicrobial agents[24] of use in areas of high prevalence may be assessed to enable the development of treatment schedules and forestall the selection of more resistant strains.

Perhaps the trends in future may be directed towards the production of a new generation antimicrobial agent that is cheap, effective, non-toxic, orally administered, and more readily acceptable in the developing countries.

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